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Markers and characteristics of human SCLC cell lines

Neuroendocrine markers, classical tumor markers, and chromosomal characteristics of permanent human small cell lung cancer cell lines *

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Summary. Permanent human small cell lung cancer (SCLC) cell lines established in our laboratory were investigated for their expression of the enzymatic neuroendocrine markers L-DOPA decarboxylase (DDC), neuron-specific enolase (NSE), and creatine kinase (CK), including its BB isoenzyme (CK-BB), the classical tumor markers carcinoembryonic antigen (CEA), the α and β subunits of human chorionic gonadotropin (α-HCG, β-HCG), and α-fetoprotein (α-FP), and their chromosomal characteristics. DDC activities were detectable in 5/6 SCLC cell lines and absent in non-SCLC. NSE levels ranged from 160 to 1422 ng/mg soluble protein and were <290 ng/mg soluble protein in non-SCLC. Activities of CK and levels of CK-BB clearly distinguished SCLC from non-SCLC with CK acticities >1000 munits/mg soluble protein and CK-BB levels >3000 ng/mg soluble protein in SCLC and <300 munits/mg soluble protein and <2000 ng/mg soluble protein in non-SCLC. CEA was detectable in 5/6 SCLC cell lines but absent in non-SCLC, and its level seemed to correlate with those of DDC, NSE, and CK. One cell line, SCLC-16H, lost some of its neuroendocrine properties and CEA after 1 year of in vitro cultivation. Generally, marker levels were low in fast growing cell lines and high in slow growing cell lines. HCG α and β subunit and α-FP were not detectable in SCLC cell lines. All SCLC cell lines examined had near diploid DNA indices and modal chromosome numbers. Double minute chromosomes and homogeneously staining regions were found in 2/5 and 4/5 SCLC cell lines respectively. With respect to chromosomal aberrations, we found a deletion of the short arm of at least one chromosome 3 in all SCLC cell lines (5/5). These data show that (1) SCLC expresses neuroendocrine markers and CEA; (2) CK is the most sensitive marker, and DDC and CEA are the most specific markers for SCLC in vitro; (3) individual marker levels correlate with each other and the in vitro malignancy of SCLC; and (4) SCLC cell lines have relatively uniform chromosomal chracteristics. Our results suggest that patients whose tumors have high levels of DDC, NSE, CK-BB, and CEA have a better prognosis than those with low marker levels. This hypothesis could be proved by comparing pairs of patients that are matched for all known prognostic parameters, in particular tumor spread, for their serum and tumor marker levels with respect to the patients' outcome and prognosis.

Key words: Small cell lung cancer – Cell lines – Neuroendocrine markers – Tumor markers – Chromosomal characteristics

Introduction

Since the 1970s, an increasing number of continuously growing small cell lung cancer (SCLC) cell lines have been established, and they provide a sound basis for mately 100 well characterized SCLC cell lines have been established, and they provide an sound basis for studying the biology of this particular type of lung cancer (Gazdar et al. 1980, 1985a; Pettengill et al. 1980; Carney et al. 1985; Baillie-Johnson et al. 1985; Bergh et al. 1985; Bepler et al. 1987).

In vitro studies have shown that SCLC expresses numerous properties of the neuroendocrine system including L-DOPA decarboxylase (DDC) (Baylin et al. 1980), bombesin-like immunoreactivity (Moody et al. 1981), neuron-specific enolase (NSE) (Marangos et al. 1982), kinase and the BB isoenzyme of creatine (CK-

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BB) (Gazdar et al. 1981) which can be easily used to distinguish SCLC from non-SCLC. Although most SCLC cell lines express all 4 markers, a subset of cell lines (approximately 30%) lack DDC and bombesin-like immunoreactivity and have lower levels of NSE. These cell lines are called variant subtype in contrast to the classic subtype of SCLC and morphologically resemble large cell lung cancer, have short population doubling times (PDT), high colony forming efficiencies in soft agarose, and are amplified for the c-*myc* proto-oncogene (Carney et al. 1985; Gazdar et al. 1985a). In vitro drug testing has revealed that the variant subtype of SCLC is radioresistant (Carney et al. 1983) but sensitive to treatment with interferon (Bepler et al. 1986). For both types of SCLC, a specific chromosomal aberration, a deletion 3p (14–23), has been described which was undetectable in non-SCLC (Whang-Peng et al. 1982a).

For the SCLC cell lines we have started whose establisment, growth properties, and morphology have been recently described (Bepler et al. 1987), we provide data here on expression of the neuroendocrine markers DDC, NSE, and CK, the tumor markers carcinoembryonic antigen (CEA), α and β subunits of human chorionic gonadotropin (HCG), and α-fetoprotein (α-FP), and their chromosomal characteristics. The marker expression is compared to non-SCLC and to the PDT as an in vitro malignancy parameter. Possible clinical implications are discussed.

Materials and methods

Cell lines. The permanent human SCLC cell lines used were SCLC-16HC, SCLC-16HV, SCLC-21H, SCLC-22H, SCLC-24H, SCLC-86M1, SCLC-86M2, and SCLC-203H; the non-SCLC cell lines of squamous cell origin were EPLC-32M1, EPLC-65H, and U-1752; of adenocarcinoma origin NCI-H23 and NCI-H125; of large cell origin LCLC-103H and U-1810; and of mesotheliomal origin MSTO-211H. Cell lines of small cell origin and cell lines EPLC-32M1, EPLC-65H, LCLC-103H, and MSTO-211H were established in our laboratory (Bepler et al. 1987). All other cell lines were obtained from Drs. Adi F. Gazdar and John D. Minna, National Cancer Institute, Bethesda, Md. USA (Bergh et al. 1981, 1985; Carney et al. 1985). Cells lines were kept continuosly growing in RPMI 1640 medium supplemented with 10% v/v fetal bovine serum (R10; both purchased from GIBCO Europe, Paisley, UK). Non-SCLC cell lines, in contrast to SCLC cell lines, grew substrate adherent and were trypsinized upon passaging (0.05% trypsin and 0.02% EDTA solution in phosphate buffered saline (PBS) without Ca^{2+} and Mg^{2+}, obtained from cell culture Boehringer, Mannheim, FRG). All cell lines expressed human isoenzymes and were free of mycoplasma contamination (Institute for Microbiology, Marburg, FRG).

Radioimmunossays and enzyme immunoassay. The radioimmunoassay (RIA) kit for NSE (γ-subunit) was obtained from Pharmacia, Uppsala, Sweden; for CK-BB, α-HCG, and β-HCG from Institut National des Radioelements, Fleurus, Belgium; and for α-FP from Diagnostic Products Corporation, Bad Nauheim, FRG. All RIA kits were based on the polyclonal double antibody technique. The solid phase enzyme immunoassay (EIA) kit for CEA determinations using a monoclonal antibody was obtained from Abbott, Irving, Tex., USA.

Evaluation of biochemical markers. The activities and concentrations of enzymatical neuroendocrine markers and classical tumor markers were assessed in cell homogenates and normal lung homogenates collected within 12 h after death. Approximately 10^7 cells were collected in logarithmic growth phase, washed twice in PBS, homogenized by sonication, centrifuged at 30000 *g* and 4 °C for 15 min, and frozen at −20 °C in 250 µl aliquots until use. The protein concentration of homogenates was determined by means of a Coomassie brilliant blue binding protein assay (Bio-Rad, Munich, FRG). DDC activity was determined as decribed by Beaven et al. (1978) and modified by Baylin et al. (1978), calculated as nanomoles CO_2 release per h and milligrams soluble protein, and expressed in units per milligram. Total CK activity was determined by standard kinetic assay as published (Deutsche Gesellschaft für Klinische Chemie 1977) and expressed in milliunits per milligram soluble protein.

Concentrations of NSE, CK-BB, CEA, α-HCG, β-HCG, and α-FP were evaluated by commercially available RIA or EIA kits according to the manufacturers' instructions and expressed in nanograms per milligram soluble protein.

Determination of the PDT. The PDT was determined during logarithmic growth phase for all SCLC cell lines in liquid culture as previously described (Bepler et al. 1987).

DNA content and chromosomal characteristics. DNA content of cell lines was evaluated by flow cytometry as described elsewhere (Bunn et al. 1983). Approximately 10^6 cells were stained with propidium iodide and the modal channel number of tumor cells in G_1 phase of the cell cycle was divided by the modal channel number of normal human diploid standard cells to obtain the DNA index. Examination of the cell lines for modal chromosome numbers and chromosomal aberrations was done by a modified Giemsa-trypsin technique (Seabright 1971). A total of 50 metaphases were evaluated for chromosome numbers and 10 for chromosomal aberrations.

Results

Expression of neuroendocrine and classical tumor markers

The expression of the neuroendocrine markers DDC, NSE, CK, and CK-BB were assessed in cell pellets and used to characterize the individual cell lines and to compare their expression among SCLC, non-SCLC, and normal lung tissue. Individual marker levels are listed in Table 1. The results were: (1) activity of DDC was detectable in 5/6 SCLC and 0/8 non-SCLC cell lines; normal lung had no detectable activity; (2) NSE levels ranged from 160 to 1422 ng/mg soluble protein in SCLC and 45 to 290 ng/mg soluble protein in non-SCLC. Levels higher than 300 ng/mg soluble protein were found in 5/6 SCLC and 0/6 non-SCLC cell lines, and normal lung was below the range of non-SCLC cell lines; (3) high total activities of CK (> 1000 munits/mg soluble protein) were measured in 6/6 SCLC (range 1056–8533 munits/mg soluble pro-

Table 1. The levels and activities of the neuroendocrine enzymatical markers L-DOPA decarboxylase (DDC), neuron-specific enolase (NSE), and creatine kinase (CK) and levels of the classical tumor marker carcinoembryonic antigen (CEA) of established permanent human lung cancer cell lines of different histologies and normal lung tissue are compared. Sonified cell lysates were used and the repective markers determined by radioimmunoassays (NSE and CK-BB), enzymatic methods (DDC and CK), and enzyme immunoassay (CEA). Marker levels are given per milligram soluble protein. The population doubling times (PDT) of individual small cell lung cancer (SCLC) cell lines are listed for comparison with the respective marker levels

Cell line	DDC	NSE	CK	CK-BB	CEA	PDT
	[units/mg]	[ng/mg]	[munit/mg]	[ng/mg]		[h]
SCLC-16HC	385	541	3367	10226	49.7	65
-16HV	< 1	160	1056	3339	< 2.0	40
SCLC-21H	< 1	598	2929	6338	< 2.0	45
SCLC-22H	300	661	4353	11479	62.2	75
SCLC-24H	205	343	2014	8286	66.9	70
SCLC-86M1	4	270	2111	7650	2.5	70
-86M2	22	214	1480	7088	4.2	70
SCLC-203H	208	1422	8533	NT[a]	> 60.0	> 200
Non-SCLC	< 1	45–290	36–261	321–1614	< 2.0	–
Normal lung	< 1	14	4	38	2.5	–
Detection limit[b]	0	2	0	1	2.0	–

[a] NT = not tested
[b] Detection limit in ng/ml

tein) and 0/5 non-SCLC cell lines (range 36–261 munits/mg soluble protein); the activity of non-SCLC cell lines, however, was higher than the activity of normal lung; and (4) the same results were obtained for CK-BB levels, i.e., the highest levels were found in SCLC cell lines (range 3339–11479 ng/mg soluble protein) without overlap with non-SCLC cell lines (range 321–1641 ng/mg soluble protein), and the levels in non-SCLC cell lines were higher than those of normal lung.

Among the classical tumor markers CEA, α- and β-HCG, and α-FP, only CEA was detectable in SCLC cell lines. Five of six cell lines examined had levels above normal lung and ranged from 2.5 to 66.9 ng/mg soluble protein. All non-SCLC cell lines had undetectable CEA levels (0/8).

Individual marker levels showed a correlation with the PDT and among each other with high levels found predominantly in slow growing cell lines (for instance SCLC-203H) and low levels in fast growing ones (for instance SCLC-21H). With respect to the DDC activity, cell line SCLC-21H belonged to the variant subtype of SCLC, whereas all other cell lines belonged to the classic subtype (Carney et al. 1985; Gazdar et al. 1985a). No significant differences were found between sublines 1 and 2 of cell line SCLC-86M, which were established after the first and second nude mouse passage respectively.

A loss of neuroendocrine differentiation was found in cell line SCLC-16H after approximately 1 year of in vitro cultivation. This cell line expressed all neuroendocrine markers and CEA up until passage 50 and thus belonged to the classic subtype of SCLC (SCLC-16HC); DDC activity and CEA levels, however, were undetectable from passage 57 on. This spontaneously developed variant subline, designated SCLC-16HV, also had lower levels of NSE, CK, and CK-BB and a shorter PDT than its classic counterpart SCLC-16HC (Table 1).

DNA content and chromosome analysis

DNA content analysis was performed in 5/6 SCLC cell lines and showed near diploid DNA indices with a single G_0/G_1 peak in all cell lines examined (Table 2). In accordance with these data, the modal chromosome number ranged from 42 to 44. Only cell line SCLC-16HV had a bimodal chromosome distribution with a major peak at 44 chromosomes (13/50 metaphases) and a minor peak at 53 chromosome (9/50 metaphases). The range of chromosome numbers in individual cell lines was narrow with cell line SCLC-16HV having the widest range. All cell lines examined (5/5) had a deletion of the short arm of at least one chromosome 3 with region 3p (14–23) always being involved as previously described by Whang-Peng et al. (1982a). Double minute chromosomes (DMs) were found in SCLC-22H and SCLC-24H and homogeneously staining regions (HSRs) in all cell lines examined except SCLC-21H.

Table 2. The grade of ploidy determined by flow cytometry and chromosomal characteristics of SCLC cell lines are listed and compared to non-SCLC. Modal chromosome number, range of chromosome number, and the incidence of double minute chromosomes (DMs) was determined in 50 metaphases of each cell line. The incidence of homogeneonsly staining regions (HSRs) and deletions of the short arm of at least one chromosome 3 per metaphase was evaluated in 10 metaphases for each cell line. The minimal region being involved in this deletion was region 14–23 as described by Whang-Peng et al. (1982a)

Cell line	DNA index	Modal chromosome number	Range of chromosome number	DMs	HSRs	Deletion 3p (14–23)
SCLC-16HC	1.05	NT*	NT	NT	NT	NT
-16HV	NT	44+53	43–56	0/50	5/10	5/10
SCLC-21H	0.98	42+43	39–44	0/50	0/10	5/9
SCLC-22H	0.98	43	40–47	1/50	1/10	4/10
SCLC-24H	0.98	42	36–45	2/50	1/10	10/10
SCLC-86M1	NT	42	40–45	0/50	1/10	10/10
-86M2	1.04	NT	NT	NT	NT	NT
Non-SCLC	>1.3	>70	–	–	–	Absent

* NT = not tested

Non-SCLC cell lines (4/4) had DNA indices above 1.3, modal chromosome numbers above 70, and lacked the deletion 3 p (14–23). Data concerning the chromosomal and DNA content characteristics are summarized in Table 2.

Discussion

The levels and activities of enzymatic neuroendocrine markers, the levels of classical tumor markers, and the chromosomal characteristics of permanent human SCLC cell lines were studied and compared among indivudual cell lines and between SCLC and non-SCLC. Our results indicate that (1) CK activity and CK-BB levels clearly distinguish SCLC from non-SCLC with no overlap between the two lung cancer entities; (2) DDC and CEA concentrations correlate with each other and appear to be specific markers for SCLC in vitro, but they are not uniformly elevated in all SCLC cell lines; (3) high NSE levels are found in all SCLC cell lines, but they overlap with levels found in non-SCLC cell lines; (4) high marker levels correlate with low in vitro malignancy as determined by the PDT; and (5) SCLC cell lines have relatively uniform chromosomal characteristics and can be distinguished from non-SCLC by their modal chromosome number and deletion of the short arm of chromosome 3.

With respect to the differential expression of biomarkers among established human lung cancer cell lines, we obtained results comparable to those published by other authors concerning the use of DDC, NSE, and CK (CK-BB) as markers for SCLC (Baylin et al. 1980; Marangos et al. 1982; Gazdar et al. 1981; Carney et al. 1985). These enzymes are characteristic markers of the neuroendocrine system whose amine precursor uptake and decarboxylation (APUD) properties were delineated by Pearse (1969; Pearse and Takor Takor 1979). Our results thus confirm the classification of SCLC as a neuroendocrine neoplasm of the lung (Gould et al. 1983).

CEA showed a strikingly differential distribution between SCLC and non-SCLC cell lines. While 5/6 SCLC cell lines had CEA levels equal or higher than 2.5 ng/mg soluble protein, all non-SCLC cell lines had levels lower than the detection limit of the assay used. All CEA positive cell lines had a detectable DDC activity, and cell line SCLC-16H lost both DDC and CEA simultaneously during prolonged in vitro cultivation. These data together with results published by Gazdar et al. (1985 b), who found CEA in 60% of classical SCLC cell lines but none of the variant SCLC cell lines and non-SCLC cell lines, indicate an easy biochemical way to distinguish the classical from the variant SCLC subtype in vitro and perhaps in vivo. Preliminary results from our recently launched immunohistochemical studies seem to confirm this result in cell lines. A considerable heterogeneity, however, exists among cells from one cell line, and the level of staining ranges from absent to very intense. The absence of detectable CEA levels in the adenocarcinoma cell lines tested is in contrast to immunohistochemical studies on adenocarcinomas and may be attributed to the low number of adenocarcinomas of the lung available for in vitro studies to date. Another possible explantation is that all adenocarcinoma cell lines available belong to the undifferentiated form of this tumor type and thus lack most of the typical adenocarcinoma features.

Since antibodies are able to distinguish at least nine different regions of the glycoprotein CEA, some of which are shared with normal cross-reacting antigens (NCA; Haggarty et al. 1986), it is of major interest to know which of these regions are recognized by the commercially available antibodies used in this study and in the study published by Gazdar et al. (1985 b). It would be intriguing for speculations on the histogenesis of lung cancer if the epitope detected by these antibodies was present on NCA-1, a glycoprotein with physicochemical and immunological properties similar to CEA, which was predominantly found in human lung and myeloid cells (Rogers 1983).

Individual marker levels correlated among SCLC cell lines, and high levels were found in slow growing cell lines and vice versa (Table 1). These data together with the previously demonstrated correlation between morphological characteristics and in vitro malignancy parameters (Bepler et al. 1987) demonstrate that SCLC has a wide degree of differentiation. Tumors with slow growth, high marker levels, and inconspicuous nucleoli are more differentiated than those with fast growth, low marker levels, and visible nucleoli. The most undifferentiated form of SCLC, which morphologically resembles large cell lung cancer and does not express the key APUD enzyme DDC and CEA as shown here, was recently introduced as a variant subtype of SCLC (Carney et al. 1985; Gazdar et al. 1985 a). Since NSE levels overlap with those of non-SCLC and DDC activities are undetectable in the variant SCLC subtype, CK-BB must be the most sensitive in vitro marker for SCLC, because even SCLC at the low scale of differentiation, i.e., the variant subtype, can easily be distinguished from non-SCLC by its high CK-BB levels.

One would expect from the data presented that SCLC patients with high marker levels in sera, strong immunohistochemical tumor staining for these markers, and morphologically inconspicuous nucleoli have a better prognosis than those with low marker levels, weak immunostaining, and visible nucleoli, provided all other parameters influencing the prognosis are taken into account. Data by Radice et al. (1982) and Carney et al. (1980) partially confirmed this assumption concerning the morphological characteristics. However, it must be remembered that the material obtained for morphological examination reveals SCLC of the oat cell type in a large proportion, which is considered an artifact due to insufficient nutrient and oxygen supply (Matthews and Gazdar 1981). The prognostic value of morphological examinations is thus doubtful. With respect to the prognostic value of marker levels in sera of patients, up-to-date data seem to contradict the above assumption that patients with well differentiated tumors, i.e., high marker levels and slow growth, have a better prognosis (Waalkes et al. 1980; Carney et al. 1982, 1984). In these studies and many others (not referenced) the authors demonstrated a correlation between the marker levels and the extent of disease or tumor burden. It is therefore not surprising that patients with high marker levels did significantly worse than those with low or normal marker levels, since tumor burden and disease extent are the most significant prognostic factors for patients suffering from SCLC and other tumors. These three markers (NSE, CK-BB, CEA) are released into the circulation upon cell death or severe cell damage which will occur more often in large tumors than in minute or small tumors. Because serum marker levels are a function of marker production, marker release, and marker clearance, we conclude that patients with similar extent of disease, tumor size, and marker clearance should be evaluated for the prognostic value of serum marker levels and hypothesize that patients with high marker levels have a better prognosis than those with low marker levels. In fact, Sehested et al. (1981) demonstrated that patients with strong CEA staining of the tumor had a better survival rate than those with absent staining. These data, however, are in contradiction to those of Goslin et al. (1983) who found a positive correlation between extent of disease and thus a poorer prognosis and positive immunohistochemical tumor staining for CEA. Further studies on a large number of cell lines and fresh tumor specimens using immunocytochemistry and immunohistochemistry together with radioimmunological methods are necessary to elucidate in detail the prognostic value of CEA and neuroendocrine markers for SCLC.

All SCLC cell lines described here had near diploid modal chromosome numbers and DNA indices. Whang-Peng et al. (1982a) found near diploid modal chromosome numbers in 5/12 SCLC cell lines, and Bunn et al. (1983) reported that 17% (15/17) of fresh tumor specimens had near diploid DNA indices. This discrepancy is probably due to the small number of cell lines examined. Our results comparing the DNA index between SCLC and non-SCLC, however, are in good agreement with literature data indicating that non-SCLC have a higher DNA content than SCLC. With respect to the 3p deletion in SCLC, we were able to confirm earlier results by Whang-Peng et al. (1982 b) who demonstrated the presence of a deletion of the short arm of at least one chromosome 3 in 50% –100% of metaphases in all SCLC cell lines and fresh tumor specimens. The minimum region involved was 14–23 and non-SCLC cell lines (5/5) did not reveal this specific chromosomal aberration (Whang-Peng et al. 1982 a). DMs and HSRs, which are thought to be the cytogenetic correlate of gene amplifications (Schimke

1984), were found in 4 SCLC cell lines but were absent in cell line SCLC-21H. This is rather surprising, since Whang-Peng and coworkers (1982b) found DMs and HSRs predominantly in the variant subtype of SCLC. SCLC-21H, however, exhibits the most malignant properties in vitro compared to the other SCLC cell lines and belongs to the variant subtype of SCLC. This may be explained by a possible loss of DMs during cultivation (Schimke 1984).

We conclude that SCLC expresses a wide range of differentiation with the variant subtype as the most undifferentiated form. Although this most undifferentiated form of SCLC resembles large cell lung cancer morphologically, it can be clearly distinguished from non-SCLC by the concentration of CK and CK-BB as well as its chromosomal characteristics. This confirms the classification of SCLC as a neuroendocrine neoplasm of the lung. DDC, CK-BB, NSE, and CEA concentrations in tumors seem to correlate with the grade of differentiation and thus perhaps the patients' prognosis and outcome. To prove this hypothesis a study should be started to compare marker levels in sera and tumor tissues between otherwise matched pairs of patients to evaluate in detail their prognostic significance for SCLC.

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